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<b>(21) International Application Number:</b> PCT/US99/13923 <b>(22) International Filing Date:</b> 21 June 1999 (21.06.99) <b>(30) Priority Data:</b> 09/100,415 20 June 1998 (20.06.98) US <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b> US 09/100,415 (CIP) Filed on 20 June 1998 (20.06.98) <b>(71) Applicant (for all designated States except US):</b> UNITED BIOMEDICAL INC. [US/US]; 25 Davids Drive, Hauppauge, NY 11788 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> WANG, Chang, Yi [US/US]; 47 Snake Hill Road, Cold Spring Harbor, NY 11724 (US). <b>(74) Agent:</b> LIN, Maria, C., H.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> SYNTHETIC SOMATOSTATIN IMMUNOGEN FOR GROWTH PROMOTION IN FARM ANIMALS  <b>(57) Abstract</b>  The invention provides peptides comprising somatostatin, or a sequence homologous to somatostatin, which is covalently linked to a helper T cell epitope and optionally to other immunostimulatory sequences. The present invention provides for the use of such peptides as immunogens to elicit the production in mammals of high titer polyclonal antibodies, which are specific to somatostatin. The peptides are expected to be useful in lowering somatostatin levels in mammals, thereby increasing growth rate and food utilization efficiency.		

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SYNTHETIC SOMATOSTATIN IMMUNOGEN  
FOR GROWTH PROMOTION IN FARM ANIMALS

FIELD OF THE INVENTION

5           This invention relates to a peptide composition that is useful as an immunogen for growth promotion in farm animals. The immunogenic peptides of the subject composition contain helper T cell epitopes (Th) which comprise multiple class II MHC binding motifs and have somatostatin at either  
10 the C- or N- terminus. The peptides, optionally, contain an invasin domain which acts as a general immune stimulator. The helper T cell epitopes and the invasin domain enable the immune response against the somatostatin self-peptide.

BACKGROUND OF THE INVENTION

15           Recent understanding of neuro-endocrine and hormonal factors involved in growth, together with rapid advances in biotechnology, have provided potential avenues for the improvement of growth rate in a variety of farm animal species. A method for animal growth promotion that modifies  
20 the regulatory effects of growth hormones through immunoneutralization is a valuable alternative to the present methods which use antibiotics, anabolic steroids, and growth hormones as growth promoters. This application of antibiotics to animal husbandry is believed to be a driving force for the  
25 selection of antibiotic resistance in certain pathogenic bacterial species and has resulted in adverse consequences in the control of human infectious disease (Witte, Science, 1998; 279:996). The application of anabolic steroids promotes growth in farm animals, but is unpopular among consumers.  
30 Steroid use is constrained in Europe both by legislation and by public opinion (Buttery and Dawson, Proc Nutr Soc, 1990; 49:459). Recombinant DNA technology has enabled the production of metabolic hormones in large quantity for direct administration to animals. Such application can promote  
35 growth. It is, however, just one of the steps that need to be taken if the somatotrophic hormones are to be used as a means

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of improving animal performance.

The principal somatotrophic hormones are: somatotropin (growth hormone, GH), somatomedin-C (insulin-like growth factor 1, IGF-1), somatocrinin (GH releasing factor, GRF) and somatostatin (GH release inhibiting factor). The major potential applications for the somatotrophic hormones are in growth and lactation, however the hormonal control of these phenomena relies upon a complex interaction between many different hormones (Spencer, Livest Prod Sci, 1985, 12:31). Thus, simple application of a single hormone may not be sufficient to enhance productivity.

It appears that GH plays two distinct roles. It has a positive effect in stimulating an increase in muscle protein synthesis (most, if not all, of which is mediated by IGF-1) and a potent catabolic effect by its ability to breakdown fats. The overall effects are an increase in the lean content of the carcass and a decrease in carcass fat, often an increase in growth, and universally an improvement in food conversion efficiency (Buttery and Dawson, Proc Nutr Soc, 1990; 49:459; and, Spencer, Reprod Nutr Develop, 1987; 27(2B):581).

The effects on growth of GH administration are not reliable for its use in practice; probably because administration of exogenous hormones ignores the sensitive interrelationship between various hormones and ignores the possible effect of elevation of plasma levels on receptor populations. Effective application of GH requires frequent injections so as to provide a continuous physiologically effective serum level of hormone. This results in increased risk of upsetting these balanced relationships and causing adverse effects.

Thus, although pure preparations of growth hormones can be made in large quantities by recombinant DNA technology, this technology does not provide the modulated mechanism for delivery that is needed for the desired effect on growth. Moreover, the use of recombinant growth hormone in food

animals, most recently in dairy cattle, has resulted in widespread consumer opposition and regulatory obstacles. The use of growth hormone to promote lean deposition in ruminants and other farm animals has not received regulatory approval within the European Economic Community. The regulatory and political situation is similar for the application of IGF-1 or GRF to promote growth in farm animal species.

As an alternative to increasing the levels of growth stimulating hormones it may prove equally, or even more, effective to remove endogenous growth inhibitors. The major inhibitor of total somatic growth is somatostatin. Somatostatin is a cyclic peptide of fourteen amino acids and its structure is conserved across species. It is synthesized as a ninety-two amino acid prosomatostatin molecule from which six peptides including somatostatin itself and a twenty-eight amino acid form of somatostatin are known to be derived (Reichlin, J Lab Clin Med, 1987; 109:320). Somatostatin inhibits the release of many gastro-intestinal hormones as well as inhibits release of GH, insulin, thyroid hormones, thereby affecting both the ability of the animal to absorb nutrients and its subsequent ability to direct these nutrients into tissue growth.

The use of a somatostatin antagonist has been found to stimulate growth in rats (Spencer et al., Life Sci, 1985, 37:27), but this kind of treatment also suffers from the drawbacks of GH, IGF-1 and GRF in that it requires daily injections. A practical alternative is the use of the immune response to induce immunoneutralization of somatostatin. The use of the immune system to promote growth may be more acceptable to consumers and regulatory agencies than direct administration of hormones or synthetic steroids. The immunoneutralization of somatostatin by vaccine was first explored in sheep by Spencer et al. (Livest prod Sci, 1983, 10:469).

In a preliminary study using twin St. Kilda lambs, active immunization against somatostatin resulted in the treated lambs growing at 176% of the rate of the control lambs

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(Spencer et al., Anim Prod, 1981, 32:376). Subsequent studies have been unable to reproduce this figure, but an improvement of 15-20% in growth rate is more usual. The somatostatin molecule is the same in all farm animal species, and it has now been shown that active immunization against somatostatin can stimulate growth in commercially important breeds of sheep (Spencer et al., Livest Prod Sci, 1983, 10:25; Laarveld et al., Can J Anim Sci, 1986, 66:77), cattle (Lawrence et al., J Anim Sci, 1986, 63: (Suppl) 215), pigs and chickens (Spencer et al., Dom Anim Endocr, 1986, 3:55). A summary of successful examples demonstrating effective immunization against somatostatin for farm animals is shown in Table 1.

In addition to stimulating growth rate and leading to a 20% reduction in rearing time (Spencer, Reprod Nutr Develop, 1987; 27(2B):581), active immunization against somatostatin also has a beneficial effect on food conversion efficiency. In addition to the saving on food by virtue of more rapid growth, the animals actually utilize their food more efficiently during the growing period (Spencer et al., Livest Prod Sci, 1983, 10:469), at least partly as a result of changes in gut motility (Fadlalla et al., J Anim Sci, 1985, 61:234; Faichney et al., Can J Anim Sci, 1985, 64(Suppl) 93).

The treatment does not have any marked effect on carcass composition (Spencer et al., 1983, *ibid.*) but there are indications that, when killed at equal weights, treated animals may be leaner. Taken all experimental data together, active immunization appears to be a powerful, safe, and effective tool to enhance growth (Spencer, Dom Anim Endocr, 1986, 3:55).

Several immunogenic forms of somatostatin have been designed and tested as reported in the literature. For example, somatostatin has been conjugated with protein carriers to enhance immunopotency. However, protein carriers are too expensive for economical use in farm animals. Further, effective immunization with somatostatin depends on the conjugation site between somatostatin and the carrier. Most if not all of the somatostatin protein carrier conjugates

were prepared by glutaraldehyde coupling, employing cross linkage between the lysine residues present on somatostatin and the carrier protein. The two lysines on somatostatin available for coupling reside within a 12-mer functional loop thus may result in significant loss of the native somatostatin structure and reduction in crossreactivity to somatostatin when such conjugates are used as vaccines.

Moreover, protein linkage to somatostatin is problematic because the majority of immune responses are directed to the carrier rather than to somatostatin (the mass of the carrier molecule(s) is much greater than that of somatostatin) and immunization with hapten carrier conjugates frequently leads to carrier-induced immune suppression (Schutz et al., J Immunol, 1985, 135:2319). Accordingly, an immune enhancer that is suitable for live stock use, inexpensive and capable of stimulating an early and strong immune response to somatostatin has been sought. This immune enhancer should avoid carrier-induced suppression.

An important factor affecting immunogenicity of a synthetic peptide for an somatostatin immunogen is its presentation to the immune system by T helper cell epitopes. Formerly, those were provided by a carrier protein with the concomitant disadvantages discussed above. These may also be supplied as hybrid polypeptides by recombinant DNA expression systems (Riggs, US 4,812,554; US 4,563,424; and Xu et al., Science in China (Series B), 1994; 37:1234). These may also be more simply and less expensively supplied by a synthetic peptide comprising the target hapten B cell site and T-helper epitopes (Th) appropriate for the host. Such peptides react with helper T-cell receptors and the class II MHC molecules, in addition to antibody binding sites (Babbitt et al., Nature, 1985, 317:359) and thus stimulate a tightly site-specific antibody response to the target antibody binding site (target site). A wholly synthetic peptide immunogen for somatostatin would enjoy the following advantages over carrier conjugates and recombinant polypeptides: the product is chemically defined for easy quality control, it is stable, no elaborate

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downstream processing is needed, no elaborate production plant is required, and the engendered immune response is site-specific so that undesirable responses such as epitopic suppression are avoided.

5 Immunogenicity of synthetic somatostatin immunogens can be optimized by (1) combining somatostatin with selected promiscuous Th sites to which the majority of a population are responsive; (2) combining somatostatin with an enlarged repertoire of Th through combinatorial chemistry and thereby  
10 accommodate to the variable immune responsiveness of a population, and (3) the stabilization of a desirable conformational feature of somatostatin by cyclic constraint.

Epitopes termed promiscuous Th evoke efficient T cell help and can be combined with B cell epitopes that by  
15 themselves are poorly immunogenic to provide potent immunogens. Well-designed promiscuous Th/B cell epitope chimeric peptides are capable of eliciting Th responses and resultant antibody responses in most members of a genetically diverse population expressing diverse MHC haplotypes.  
20 Promiscuous Th can be provided by specific sequences derived from potent immunogens including measles virus F protein and hepatitis B virus surface antigen. Many known promiscuous Th have been shown to be effective in potentiating a poorly immunogenic peptide corresponding to the decapeptide hormone  
25 LHRH (US 5,759,551).

Potent Th epitopes range in size from approximately 15-30 amino acid residues in length, often share common structural features, and may contain specific landmark sequences. For example, a common feature is amphipathic  
30 helices, which are alpha-helical structures with hydrophobic amino acid residues dominating one face of the helix and with charged and polar residues dominating the surrounding faces (Cease et al., Proc Natl Acad Sci USA, 1987; 84: 4249-4253). Th epitopes frequently contain additional primary amino acid  
35 patterns such as a Gly or charged residue followed by two to three hydrophobic residues, followed in turn by a charged or polar residue. This pattern defines what are called Rothbard

sequences. Also, Th epitopes often obey the 1, 4, 5, 8 rule, where a positively charged residue is followed by hydrophobic residues at the fourth, fifth and eighth positions after the charged residue. Since all of these structures are composed of common hydrophobic, charged and polar amino acids, each structure can exist simultaneously within a single Th epitope (Partidos et al., J Gen Virol, 1991; 72:1293). Most, if not all, of the promiscuous T cell epitopes fit at least one of the periodicities described above. These features may be incorporated into the designs of idealized artificial Th sites, including combinatorial Th epitopes. In regard to the design of combinatorial Th sites, lists of variable positions and preferred amino acids are available for MHC-binding motifs (Meister et al., Vaccine, 1995; 13:581-591); and, a method for producing combinatorial Th has been disclosed for library peptides termed structured synthetic antigen library or SSAL (Wang et al., WO 95/11998). Thus, the 1,4,5,8 rule can be applied together with combinatorial MHC-binding motifs in the assignment of positions for the invariant and degenerate sites of an SSAL and for the selection of residues for these sites, so as to vastly enlarge the range of immune responsiveness to an artificial Th (WO 95/11998).

Peptide immunogens are generally more flexible than proteins and tend not to retain any preferred structure. Therefore it is useful to stabilize a peptide immunogen by the introduction of cyclic constraints. A correctly cyclized peptide immunogen can mimic and preserve the conformation of the targeted epitope and thereby evoke antibodies with cross-reactivities on that site on the authentic molecule (Moore, Chapter 2 in *Synthetic Peptides A User's guide*, ed Grant, WH Freeman and Company: New York, 1992, pp 63-67).

Peptide immunogens that have been designed with the peptide technologies and peptide design elements discussed above, i.e., design of promiscuous potent Th epitopes, Th SSAL combinatorial peptides, and cyclic constraint, are the basis for effective synthetic somatostatin immunogens. Such peptides are preferred for their presentation of the

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somatostatin by optimized positioning and cyclization, and for broadly reactive Th responsiveness. Hence, it has been found that peptides containing particular structural arrangements of a Th epitope alone or linked to a general immune enhancer, e.g., an invasin domain (US 5,759,551) and somatostatin in its intact form where the functional site within the 12 mer loop structure is not disturbed (as target antigen), are effective in stimulating the production of antibodies against somatostatin.

### SUMMARY OF THE INVENTION

The present invention relates to an immunogenic peptide composition comprising synthetic peptides, which are capable of inducing antibodies against somatostatin that lead to the suppression of somatostatin levels, promote growth and improve food conversion efficiency in farm animals. In particular, peptides of this invention have a Th epitope linked to a carboxyl- or amino- terminal somatostatin (SEQ ID NO:1) or a peptide analog of somatostatin. Optionally, the peptides have an invasin domain (SEQ ID NO:2) as a general immune stimulator. These peptides are effective as immunogens, capable of increasing serum growth hormone level in immunized hosts to promote daily weight gain in farm animals.

Another aspect of this invention provides an antigenic composition comprising an immunologically effective amount of a peptide composition in accordance with this invention and one or more pharmaceutically acceptable vaccine formulations and instructions for dosage such that immunotherapeutic antibodies directed against the targeted somatostatin site are generated. Such peptide compositions are useful for growth promotion in farm animals.

A further aspect of the invention relates to a method for increasing circulating somatotrophic hormone levels in a mammal by administering one or more of the subject peptides to the mammal for a time and under conditions sufficient to induce functional antibodies directed against

said somatostatin.

Yet another aspect of the invention relates to an immunogenic synthetic peptide of about 30 to about 90 amino acids which contains a helper T cell (Th) epitope, somatostatin (SEQ ID NO:1) or a peptide analog of somatostatin, spacers to separate the immunogenic domains and optionally general immunostimulatory sites, for example, an invasin domain (SEQ ID NO:2). These three immunogenic domain elements of the peptide and spacer can be covalently joined in any order provided that either the immunoreactivity of the peptide hapten is substantially preserved or that immunoreactivity to the somatostatin self-peptide can be generated.

#### DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to a novel peptide composition for the generation of high titer polyclonal antibodies with specificity for somatostatin. The high site-specificity of the peptide composition minimizes the generation of antibodies that are directed to irrelevant sites on carrier proteins. Therefore, the invention is further directed to an effective method for the growth promotion in farm animals.

Somatostatin (SEQ ID NO:1) is a short cyclized peptide hormone which, by itself is non-immunogenic, more so for being a self-antigen. This short peptide can be immunopotentiated by chemical coupling to a carrier protein, for example, keyhole limpet hemocyanin (KLH) or by fusion to a carrier polypeptide through recombinant DNA expression, for example, hepatitis B surface antigen. Major deficiencies of such "somatostatin-carrier" vaccines is that the largest portion of antibodies generated by the combinations are the non-functional antibodies directed against the carrier protein or polypeptide and the potential for epitopic suppression. The immunogens of the present invention are wholly synthetic peptides which minimize the generation of irrelevant antibodies to elicit an immune response more focused to

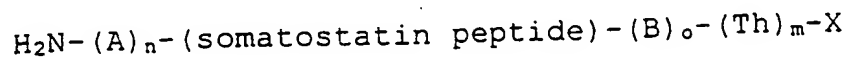
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somatostatin. However, because somatostatin is a non-immunogenic T cell-dependent antigen, it is completely dependent on extrinsic Th epitopes for immunogenicity. These are provided for the peptides of the invention as covalently linked promiscuous Th epitopes. The immunogens of the invention are all of site-specific immunoreactivity to provide for effective growth promotion in livestock.

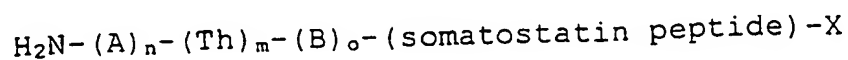
Specific examples are provided in the present invention as embodiments of the peptides of the invention. These examples provide for the linkage of synthetic immunostimulatory elements to the somatostatin peptide such that potent somatostatin-reactive antibodies are generated, in a genetically diverse host population. These antibodies, in turn, lead to inhibition of the function of somatostatin, thus resulting in an effective growth promotion for livestock.

For active immunization, the term "immunogen" referred to herein relates to a peptide composition which is capable of inducing antibodies against somatostatin, leading to inhibition or suppression of somatostatin levels in a mammal. The peptide composition of the present invention includes peptides which contain promiscuous helper T cell epitopes (Th epitopes). The peptides are covalently attached to the somatostatin peptide, with a spacer (e.g. Gly-Gly), so as to be adjacent to either the N- or C-terminus of the target somatostatin peptide, in order to evoke efficient antibody responses. The immunogen may also comprise a generalized immunostimulatory element, for example, a domain of an invasin protein from the bacteria *Yersinia* spp (Brett et al., *Eur J Immunol*, 1993, 23: 1608-1614) (SEQ ID NO:2). The invasin domain is attached through a spacer to a Th peptide.

The peptides of this invention can be represented by the formulas:



or



wherein

H<sub>2</sub>N is the N-terminal  $\alpha$ -NH<sub>2</sub> of the peptide conjugate,

each A is independently an amino acid or a general immunostimulatory sequence;

5 each B is chosen from the group consisting of amino acids, -NHCH(X)CH<sub>2</sub>SCH<sub>2</sub>CO-, -NHCH(X)CH<sub>2</sub>SCH<sub>2</sub>CO( $\epsilon$ -N)Lys-, -NHCH(X)CH<sub>2</sub>S-succinimidyl( $\epsilon$ -N)Lys-, and -NHCH(X)CH<sub>2</sub>S-(succinimidyl)-;

10 each Th is independently a sequence of amino acids that comprises a helper T cell epitope, or an immune enhancing analog or segment thereof;

somatostatin peptide is somatostatin or a crossreactive and immunologically functional analog thereof;

X is an amino acid  $\alpha$ -COOH or  $\alpha$ -CONH<sub>2</sub>;

15 n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

20 The peptide immunogen of the present invention comprises from about 20 to about 100 amino acid residues, preferably from about 25 to about 80 amino acid residues and more preferably from about 25 to about 65 amino acid residues.

25 When A is an amino acid or a general immunostimulatory element, e.g., Inv, it can be covalently linked to either the N-terminal of the peptide immunogen as shown by the formulas, or to the C-terminal (not shown).

30 When A is an amino acid, it can be any non-naturally occurring or any naturally occurring amino acid. Non-naturally occurring amino acids include, but are not limited to,  $\beta$ -alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine,  $\gamma$ -amino butyric acid, homoserine, citrulline and the like. Naturally-occurring amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine,

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isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Moreover, when m is greater than one, and two or more of the A groups are amino acids, then each amino acid may be independently the same or different.

When A is an invasin domain, it is an immune stimulatory epitope from the invasin protein of a *Yersinia* species. This immune stimulatory property results from the capability of this invasin domain to interact with the  $\beta 1$  integrin molecules present on T cells, particularly activated immune or memory T cells. The specific sequence for an invasin domain found to interact with the  $\beta 1$  integrins has been described by Brett et al (*Eur J Immunol*, 1993). A preferred embodiment of the invasin domain (Inv) for linkage to a promiscuous Th epitope has been previously described in US 5,759,551 and is incorporated herein by reference. The said Inv domain has the sequence:

Thr-Ala-Lys-Ser-Lys-Lys-Phe-Pro-Ser-Tyr-Thr-Ala-Thr-Tyr-Gln-Phe  
(SEQ ID NO:2)

or is an immune stimulatory homologue thereof from the corresponding region in another *Yersinia* species invasin protein. Such homologues thus may contain substitutions, deletions or insertions of amino acid residues to accommodate strain to strain variation, provided that the homologues retain immune stimulatory properties.

In one embodiment, n is 1 and A is  $\alpha$ -NH<sub>2</sub>. In another embodiment, n is 4 and A is  $\alpha$ -NH<sub>2</sub>, an invasin domain (Inv), glycine and glycine, in that order.

B is a spacer and is an amino acid which can be naturally occurring or the non-naturally occurring amino acids as described above. Each B is independently the same or different.. The amino acids of B can also provide a spacer, e.g., Gly-Gly, between the promiscuous Th epitope and the somatostatin peptide (e.g., SEQ ID NO:1) and crossreactive and functional immunological analogs thereof. In addition to physically separating the Th epitope from the B cell epitope,

i.e., the somatostatin peptide and immunological analogs thereof, the Gly-Gly spacer can disrupt any artifactual secondary structures created by the joining of the Th epitope with the somatostatin peptide and crossreactive and functional immunological analogs thereof and thereby eliminate interference between the Th and/or B cell responses. The amino acids of B can also form a spacer which acts as a flexible hinge that enhances separation of the Th and IgE domains. Examples of sequences encoding flexible hinges are found in the immunoglobulin heavy chain hinge region. Flexible hinge sequences are often proline rich. One particularly useful flexible hinge is provided by the sequence Pro-Pro-Xaa-Pro-Xaa-Pro (SEQ ID NO:3), where Xaa is any amino acid, and preferably aspartic acid. The conformational separation provided by the amino acids of B permits more efficient interactions between the presented peptide immunogen and the appropriate Th cells and B cells and thus enhances the immune responses to the Th epitope and the antibody-eliciting epitope and their crossreactive and functional immunological analogs thereof.

Th is a sequence of amino acids (natural or non-natural amino acids) that comprises a Th epitope. A Th epitope can consist of a continuous or discontinuous epitope.

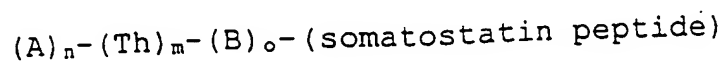
Hence not every amino acid of Th is necessarily part of the epitope. Accordingly, Th epitopes, including analogs and segments of Th epitopes, are capable of enhancing or stimulating an immune response to the somatostatin peptide and immunological analogs thereof. Th epitopes that are immunodominant and promiscuous are highly and broadly reactive in animal and human populations with widely divergent MHC types (Partidos et al., 1991; US 5,759,551). The Th domain of the subject peptides has from about 10 to about 50 amino acids and preferably from about 10 to about 30 amino acids. When multiple Th epitopes are present (i.e.,  $m \geq 2$ ), then each Th epitope is independently the same or different. Th segments are contiguous portions of a Th epitope that are sufficient to enhance or stimulate an immune response to the somatostatin

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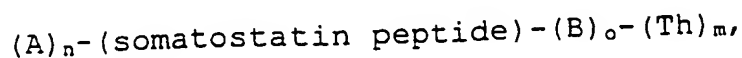
peptide (SEQ ID NO:1) and immunological analogs thereof.

Th epitopes of the present invention include those derived from foreign pathogens including but not limited to, as examples, hepatitis B surface and core antigen helper T cell epitopes (HB<sub>s</sub> Th and HB<sub>c</sub> Th), pertussis toxin helper T cell epitopes (PT Th), tetanus toxin helper T cell epitopes (TT Th), measles virus F protein helper T cell epitopes (MV<sub>F</sub> Th), *Chlamydia trachomatis* major outer membrane protein helper T cell epitopes (CT Th), diphtheria toxin helper T cell epitopes (DT Th), *Plasmodium falciparum* circumsporozoite helper T cell epitopes (PF Th), *Schistosoma mansoni* triose phosphate isomerase helper T cell epitopes (SM Th), and *Escherichia coli* TraT helper T cell epitopes (TraT Th). The pathogen-derived Th selected here as representative examples of promiscuous Th were listed as SEQ ID NOS:2-9 and 42-52 in US 5,759,551; as CT Th P11 in Stagg et al., *Immunology*, 1993; 79:1-9; and as HBc peptide 50-69 in Ferrari et al., *J Clin Invest*, 1991; 88: 214-222; and incorporated herein by reference. Further, Th epitopes include idealized artificial Th (e.g., SEQ ID NOS:14) and artificial SSAL Th (e.g., SEQ ID NOS:7,30,31). Peptides comprising SSAL Th are produced simultaneously in a single solid-phase peptide synthesis in tandem with somatostatin and other sequences. Th sites also include functional immunological analogs. Functional Th analogs include immune-enhancing analogs, crossreactive analogs and segments of any of these Th epitopes. Functional Th analogs further include conservative substitutions, additions, deletions and insertions of from one to about 10 amino acid residues in the Th epitope which do not essentially modify the Th-stimulating function of the Th epitope.

The synthetic peptides of this invention, as described by the formulas



or



have the Th epitope covalently attached through spacer B to either the N terminus or C terminus of the somatostatin peptide and crossreactive and functional immunological analogs thereof.

5 Crossreactive and functional immunological analogs of the somatostatin peptide (e.g., SEQ ID NO:1) according to the invention, may further comprise conservative substitutions, additions, deletions, or insertions of from one to about four amino acid residues provided that the peptide  
10 analogs are capable of eliciting immune responses crossreactive with the somatostatin peptides. The conservative substitutions, additions, and insertions can be accomplished with natural or non-natural amino acids as defined herein.

15 Preferred peptide immunogens of this invention are the peptides containing the somatostatin peptides or crossreactive and functional immunological analogs thereof; a spacer (e.g., Gly-Gly); a Th epitope that is an HB<sub>s</sub> Th (SEQ ID NO:15), HB<sub>c</sub> Th (SEQ ID NO:4), MV<sub>F</sub> Th (SEQ ID NOS:21,29), PT Th  
20 (SEQ ID NO:6), TT Th (SEQ ID NO:5); CT Th (SEQ ID NO:27), DT Th (SEQ ID NO:28), an artificial Th (e.g., SEQ ID NOS:7,14,30,31) or an analogue thereof; and, optionally, an Inv domain (SEQ ID NO:2) or analog thereof.

25 Peptide compositions which contain cocktails of the subject peptide immunogens with two or more of the Th epitopes may enhance immunoefficacy in a broader population and thus provide an improved immune response to the somatostatin peptide.

The peptide immunogens of this invention can be made by chemical synthesis methods which are well known to the  
30 ordinarily skilled artisan. See, for example, Fields et al., Chapter 3 in *Synthetic Peptides: A User's Guide*, ed. Grant, W. H. Freeman & Co., New York, NY, 1992, p. 77. Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the  $\alpha$ -NH<sub>2</sub> protected by either t-  
35 Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer

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Model 430A or 431. Preparation of peptide constructs comprising SSALs for Th epitopes can be accomplished by providing a mixture of alternative amino acids for coupling at a given variable position.

- 5           After complete assembly of the desired peptide immunogen, the resin is treated according to standard procedures to cleave the peptide from the resin and deblock the functional groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well known to one of ordinary skill in the art.

- The subject immunogen may also be polymerized. Polymerization can be accomplished for example by reaction between glutaraldehyde and the  $-NH_2$  groups of the lysine residues using routine methodology. By another method, the synthetic

"A-Th-spacer-(somatostatin peptide)"

or

- 20           "(somatostatin peptide)-spacer-(Th)<sub>m</sub>-A"
- immunogen can be polymerized or co-polymerized by utilization of an additional cysteine added to the N-terminus of the synthetic "A-Th-spacer-(somatostatin peptide) or "(somatostatin peptide)-spacer-(Th)<sub>m</sub>-(A)<sub>n</sub>" immunogen. The subject immunogen may also be prepared as a branched polymer through synthesis of the desired peptide construct directly onto a branched poly-lysyl core resin (Wang, et al., Science, 1991; 254:285-288).

- Alternatively, the longer synthetic peptide immunogens can be synthesized by well known recombinant DNA techniques. Any standard manual on DNA technology provides detailed protocols to produce the peptides of the invention. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated into a nucleic acid sequence, and preferably using optimized codon usage for the organism in which the gene will be expressed. Next, a synthetic gene is made, typically by synthesizing overlapping

oligonucleotides which encode the peptide and any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and recombinants are obtained and characterized. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

The efficacy of the peptide composition of the present invention can be established by injecting an animal, for example, rats, with an immunogenic composition comprising peptides of the invention, e.g., SEQ ID NOS:8-13, 16-20, 22 followed by monitoring the humoral immune response to the somatostatin and crossreactive and functional immunological homologues thereof, as detailed in the Examples.

Another aspect of this invention provides a peptide composition comprising an immunologically effective amount of one or more of the peptide immunogens of this invention in a pharmaceutically acceptable delivery system. Accordingly, the subject peptides can be formulated as a peptide composition using adjuvants, pharmaceutically-acceptable carriers or other ingredients routinely provided in peptide compositions. Among the ingredients that can be used in this invention are adjuvants or emulsifiers including alum, incomplete Freund's adjuvant, liposyn, saponin, squalene, L121, emulsigen monophosphoryl lipid A (MPL), QS21, ISA51, ISA35, ISA206 and ISA 720 as well as the other efficacious adjuvants and emulsifiers. The formulations include formulations for immediate release and/or for sustained release, and induction of systemic immunity, which may be accomplished by, for example, immunogen entrapment by or coadministration with microparticles. Such formulations are readily determined by one of ordinary skill in the art. The present immunogens can be administered by any convenient route including subcutaneous, oral, intramuscular, or other parenteral or enteral route. Similarly the immunogens can be administered as a single dose or multiple doses. Immunization schedules are readily determined by the ordinarily skilled artisan.

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The peptide composition of the instant invention contain an effective amount of one or more of the peptide immunogens of the present invention and a pharmaceutically acceptable carrier. Such a composition in a suitable dosage unit form generally contains about 0.5  $\mu$ g to about 1 mg of the immunogen per kg body weight. When delivered in multiple doses, it may be conveniently divided into an appropriate amount per dosage unit form. For example, an initial dose, e.g. 0.2-2.5 mg; preferably 1 mg, of immunogen represented as a peptide composition of the present invention, is to be administered by injection, preferably intramuscularly, followed by repeat (booster) doses. Dosage will depend on the age, weight and general health of the animal as is well known in the vaccine and therapeutic arts.

The immune response to synthetic somatostatin peptide immunogens can be improved by delivery through entrapment in or on biodegradable microparticles of the type described by O'Hagan et al. (*Vaccine*, 1991; 9: 768-771). The immunogens can be encapsulated with or without an adjuvant in biodegradable microparticles, to potentiate immune responses, and to provide time-controlled release for sustained or periodic responses, and for oral administration, (O'Hagan et al, 1991; and, Eldridge et al., 1991; 28: 287-294).

Specific peptide immunogens and compositions are provided in the following examples to illustrate the invention. These examples are for purpose of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

#### EXAMPLE 1

##### TYPICAL METHODS TO SYNTHESIZE SOMATOSTATIN PEPTIDE CONSTRUCTS

Peptides listed in Tables 2 and 3 were synthesized individually by the Merrifield solid-phase synthesis technique on Applied Biosystems automated peptide synthesizers (Models 430, 431 and 433A) using Fmoc chemistry. Preparation of peptide constructs comprising structured synthetic antigen

libraries (SSALs), e.g., artificial Th site termed "1,4,9 PALINDROMIC" (SEQ ID NO:7), can be accomplished by providing a mixture of the desired amino acids for chemical coupling at a given position as specified in the design. After complete  
5 assembly of the desired peptide, the resin was treated according to standard procedure using trifluoroacetic acid to cleave the peptide from the resin and deblock the protecting groups on the amino acid side chains. For cyclic peptide, the  
10 cleaved peptide was dissolved in 15% DMSO in water for 48 hrs to facilitate intradisulfide bond formation between cysteines.

The cleaved, extracted and washed peptides were purified by HPLC and characterized by mass spectrometry and reverse phase HPLC.

Peptides marked by "b" in the peptide code column  
15 were synthesized as target antigenic peptides in tandem with Th sites as shown. Th sites used include, for example, the HBs Th taken from hepatitis B virus (SEQ ID NO:15), and the novel artificial Th site termed "1,4,9 PALINDROMIC" (SEQ ID NO:7). Peptides marked by "c" are variants of the "b"  
20 constructs synthesized in tandem with the Inv domain immunostimulatory peptide (SEQ ID NO:2). Peptides marked by "d" are the reversal of the "b" constructs (e.g., somatostatin-Th) and peptides marked by "e" are the reversal of the "c" constructs (e.g., somatostatin-Th-Inv). The "b",  
25 "c", "d" and "e" constructs were synthesized with gly-gly spacers for separation of the target antigenic site from the Th site, and separation of the Th from the Inv immunostimulatory site.

30

## EXAMPLE 2

### TYPICAL METHODS TO EVALUATE IMMUNOGENICITY OF SOMATOSTATIN PEPTIDES

Somatostatin Peptide immunogens (e.g., SEQ ID NOS:8-  
13,16-20,22 and 24 as shown in Tables 2 and 3) were evaluated  
35 on groups of 4 or 5 rats as specified by the experimental immunization protocol outlined below and by serological assays

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for determination of immunogenicity.

Standard Experimental Design:

Immunogens: (1) individual peptide immunogen; or  
(2) mixtures comprising equal molar peptide

5 immunogens as specified in each example.

Dose: 100 µg in 0.5 mL per immunization unless  
otherwise specified

Route: intramuscular unless otherwise specified

Adjuvants: (1) Freund's Complete Adjuvant (CFA)/

10 Incomplete Adjuvant (IFA); or

(2) 0.4% Alum (Aluminum hydroxide);

CFA/IFA groups received CFA week 0, IFA in  
subsequent weeks. Alum groups received same formulations for  
all doses

15 Dose Schedule: 0, 2 and 4 weeks; 0, 3, and 6 weeks or  
otherwise specified.

Bleed Schedule: weeks 0, 3, 6 and 8 or otherwise specified

Species: Sprague-Dawley rats

Group Size: 4 or 5 rats/group

20 Assay: specific ELISAs for each immune serum's anti-peptide  
activity, solid-phase substrate was the cyclized somatostatin  
peptide (SEQ ID NO:1).

Blood was collected and processed into serum, and  
stored prior to titering by ELISA with the target antigenic  
25 peptides.

Anti-somatostatin antibody activities were  
determined by ELISAs (enzyme-linked immunosorbent assays)  
using 96-well flat bottom microtiter plates which were coated  
with the cyclized somatostatin peptide (SEQ ID NO:1) as  
30 immunosorbent. Aliquots (100 µL) of the peptide immunogen  
solution at a concentration of 5 µg/mL were incubated for 1  
hour at 37°C. The plates were blocked by another incubation at  
37°C for 1 hour with a 3% gelatin/PBS solution. The blocked  
plates were then dried and used for the assay. Aliquots (100  
35 µL) of the test immune sera, starting with a 1:100 dilution in  
a sample dilution buffer and ten-fold serial dilutions

thereafter, were added to the peptide coated plates. The plates were incubated for 1 hour at 37°C.

The plates were washed six times with 0.05% PBS/Tween® buffer. 100 µL of horseradish peroxidase labeled goat-anti-species specific antibody was added at appropriate dilutions in conjugate dilution buffer (Phosphate buffer containing 0.5M NaCl, and normal goat serum). The plates were incubated for 1 hour at 37°C before being washed as above.

Aliquots (100 µL) of o-phenylenediamine substrate solution were then added. The color was allowed to develop for 5-15 minutes before the enzymatic color reaction was stopped by the addition of 50 µL 2N H<sub>2</sub>SO<sub>4</sub>. The A<sub>492nm</sub> of the contents of each well was read in a plate reader. ELISA titers were calculated based on linear regression analysis of the absorbances, with cutoff A<sub>492nm</sub> set at 0.5. This cutoff value was rigorous as the values for diluted normal control samples run with each assay were less than 0.15.

Th peptide-based ELISAs. The Th peptide based ELISAs were performed essentially the same as the somatostatin ELISA described herein above except for the antigen coating steps, where microtiter wells were coating for 1 hr at 37° with the designated individual Th peptide derived from its corresponding somatostatin vaccine construct (e.g., peptides with SEQ ID NOS:14 and 15) at 5 µg/mL.

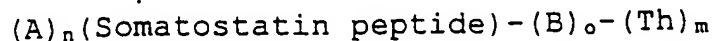
### EXAMPLE 3

#### IMMUNOGENICITY STUDIES OF SOMATOSTATIN ANTIGENIC PEPTIDES INCORPORATING VARIOUS IMMUNOSTIMULATORY ELEMENTS

The somatostatin peptide immunogens shown in Table 2 illustrate variations of the peptides of this invention represented by the formulas:



or



wherein:

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A is an amino acid,  $\alpha\text{NH}_2$ , or Inv (SEQ ID NO:2);

when A is an amino acid or Inv it can be linked to either the N-terminal or the C-terminal;

B is glycine;

5 Th is a helper T cell epitope derived from foreign pathogens, e.g., HBC<sub>50-69</sub>Th (SEQ ID NO:4), TT<sub>615-631</sub>Th (SEQ ID NO:5), PT<sub>149-176</sub>Th (SEQ ID NO:6), or and artificial Th e.g., 1,4,9 PALIDROMIC Th (SEQ ID NO:7);

n is 1, m is 1 and o is 2

10 These peptides were synthesized among others and immune sera were generated for immunogenicity evaluation. Most of the peptides shown in Table 2, incorporating various forms and orientations of Th epitopes, elicited high titer somatostatin-specific antibodies in the immunized hosts. In contrast, the somatostatin peptide (p1348a, SEQ ID NO:1) 15 lacking Th was devoid of immunogenicity. However, certain Th are to be preferred over others. For example, in Table 2, p2134b (SEQ ID NO:8) having HBC Th (SEQ ID NO:4), p2384b (SEQ ID NO:20) having SynTh(1,2,3) (SEQ ID NO:14), an artificial Th 20 site, and p2138b (SEQ ID NO:10) having PT<sub>149-176</sub> Th (SEQ ID NO:6) are more immunogenic than p2135b (SEQ ID NO:9) having TT Th (SEQ ID NO:5) and all of these are preferable to p2136b (SEQ ID NO:24) having CTA8 Th (SEQ ID NO:23) which is scarcely immunogenic at all. Also, for optimum immunogenicity, the 25 orientation of the Th site to the somatostatin target site must be specified. Compare the kinetics of the antibody response for p2253b (SEQ ID NO:11) to p2255d (SEQ ID NO:12).

It is preferable to place 1,4,9 PALINDROMIC Th (SEQ ID NO:7) on the C-terminus of somatostatin. From the comparison of 30 p1344b (SEQ ID NO:16) to p1349b (SEQ ID NO:22), the better placement for MV<sub>F258-277</sub> (SEQ ID NO:21) is on the N-terminus of somatostatin. It is clear that the selection and arrangement of each Th site must be specified for the preferred peptides of the invention.

35 For the somatostatin peptides shown in Table 3,

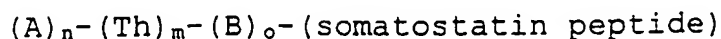
where somatostatin constructs comprising a promiscuous Th epitope were already found to be immunogenic, attachment of Inv to the "Th" constructs, can improve the immunogenicity of the somatostatin peptide. The comparisons of immunogenicities for p1344b (SEQ ID NO:16) with p1343c (SEQ ID NO:17) and p1346b (SEQ ID NO:18) with p1345c (SEQ ID NO:19) shows that the addition of the Inv domain (SEQ ID NO:2) to the N-terminus of the Th constructs improved immunogenicity in terms of percentage of responding animals, in the intensity of the somatostatin-specific antibody titer, and in the longevity of that antibody response (>35 weeks). However, the comparison of immunogenicities of p2134b (SEQ ID NO:8) with p2134c (SEQ ID NO:25) and with p2134d (SEQ ID NO:26) illustrates that in combination with particular Th sites Inv may not be immunostimulatory, and that a particular orientation on the N-terminus is preferred over the C-terminus orientation for the combination of Inv with the HBc Th site (SEQ ID NO:4). For the example of p2135e (SEQ ID NO:13), the combination of Inv with TT<sub>615-631</sub> Th (SEQ ID NO:5) on the C-terminus did result in an effective immunogen. Thus, the addition of Inv and the orientation of Th and Inv must be specified for the preferred peptides of the invention.

For constructs having potent site-directed immunogenicity for somatostatin, the antibody titers directed at the immunostimulatory elements, e.g., Inv-MVF<sub>258-277</sub> Th of p1343c (SEQ ID NO:17) and KKK-HBs<sub>19-32</sub> Th-GG of p1346b (SEQ ID NO:18) from Table 3, were <1 Log<sub>10</sub> in comparison to those of >3 Log<sub>10</sub> for somatostatin. Thus, the immune response generated by the synthetic peptides of the present invention were directed almost exclusively to the somatostatin target site.

#### EXAMPLE 4

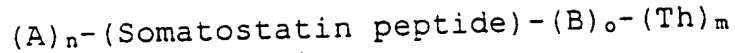
##### ADDITIONAL ANTIGENIC PEPTIDES OF THE INVENTION

Somatostatin peptide antigens illustrating variations of the peptides of this invention represented by the formulas:



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or



wherein:

5           Th is a helper T cell epitope derived from any of the foreign pathogens as shown in Table 4; or, an helper T cell epitope from any of the artificial Th epitopes as shown in Table 5;

10           A is an amino acid,  $\alpha\text{NH}_2$ , or an invasin domain (SEQ ID NO:2);

          when A is an amino acid or Inv it can be linked to either the N-terminus or the C-terminus;

          B is glycine;

          n is 1, m is 1 and o is 2

15           are synthesized and immune sera generated.

Table 1

Immunization against Somatostatin for Growth Promotion

Species	Growth as percent of controls (%)	References
Sheep (twin)	176	Spencer et al., 1981
Sheep	125	Spencer et al., 1983
Pig	118	Spencer et al., 1983
Cattle	118	Lawrence et al., 1986
Chicken	115	Spencer et al., 1986

Carrier protein-somatostatin conjugates were used as the  
5 vaccines for immunization.

Table 2  
Immunogenicity of Somatostatin Antigenic Peptides Incorporating Various Th

Peptide Code	Description of Antigenic Peptides	Amino Acid Sequence of T Helper Peptide	Adjuvant	Immunogenicity		
				WPI	Responding (n=4)	Log <sub>10</sub> ELISA <sub>b</sub> Titer <sup>a</sup>
p1348a	Somatostatin <sup>a</sup> (Seq. ID No.1)		0.4% Alum (0, 2, 4 WPI)	6	0	-
p2134b	HBC <sub>50-69</sub> - GG - Somatostatin (Seq. ID No.8)	SDFPSPVRDLIDTASALYRE (Seq. ID No.4)	0.4% Alum (0, 2, 4 WPI)	6	100	3.22
p2384b	Syn Th(1,2,4)-GG-Somatostatin (Seq. ID No.20)	KKKIITIRIITITID (Seq. ID No.14)	0.4% Alum	8	100	3.18
p2135b	TT <sub>45-63</sub> - GG - Somatostatin (Seq. ID No.9)	WVRDIIDFTNESSQKT (Seq. ID No.5)	0.4% Alum (0, 2, 4 WPI)	8	50	2.518
p2136b	CTA <sub>106-130</sub> -GG- Somatostatin (Seq. ID No.24)	ALNIWDRFDVFTLGATSGYLKGN (Seq. ID No.23)	0.4% Alum (0, 2, 4 WPI)	4	0	-
p2138b	PT <sub>149-176</sub> - GG - Somatostatin (Seq. ID No.10)	KKLRLLMIYMSGLAVRVHVSKEQYDY (Seq. ID No.6)	0.4% Alum (0, 2, 4 WPI)	6	25	2.437
p2253b	1, 4, 9 PALINDROMIC Th - GG - Somatostatin (Seq. ID No.11)	ISEIKGVIVHKIEGI MT RT TRM TM L L V (Seq. ID No.7)	0.4% Alum (0, 2, 4 WPI)	8	75	2.349
p2255d	Somatostatin -GG-1, 4, 9 PALINDROMIC Th (Seq. ID No.12)	ISEIKGVIVHKIEGI MT RT TRM TM L L V	0.4% Alum (0, 3, 6 WPI)	5	25	2.384
p1344b	MVF <sub>258-277</sub> - GG -Somatostatin (Seq. ID No.16)	GILES RGIKARITHVDTESY (Seq. ID No.21)	CFA/IFA 0, 3, 6	8	100	3.33
p1349	Somatostatin - GG - MVF <sub>258-277</sub> (Seq. ID No.22)	GILES RGIKARITHVDTESY (Seq ID NO:21)	CFA/IFA 0, 3, 6	5	0	-
				8	40	2.782

<sup>a</sup>Sequence of Somatostatin: AGCKNFEWKTFTSC

<sup>b</sup>Test results for pooled sera from ELISA-reactive animals.

Table 3.  
Improvement on Immunogenicity of Somatostatin Antigenic Peptides with the Attachment of an Invasin Domain

Peptide Code	Description of Antigenic Peptides Seq. ID No.	Amino Acid Sequence of T Helper Peptide Seq. ID No.	Adjuvant	Immunogenicity		
				WPI	% responder	Log <sub>10</sub> ELISA Titer of Responder <sup>c</sup>
p1344b	MVFT <sub>258-277</sub> - GG- Somatostatin <sup>a</sup> (Seq. ID No.16)	GILES <sup>a</sup> RGIKARITHVDTE SY (Seq. ID No.21)	CFA/IFA (0, 3, 6 WPI)	5	40	2.745
				8	80	3.111
				10	100	2.917
				12	80	3.141
				35	40	2.409
p1343c	Inv <sup>b</sup> - MVFT <sub>258-277</sub> - GG - (Seq. ID No.17)	GILES <sup>a</sup> RGIKARITHVDTE SY (Seq. ID No.21)	CFA/IFA (0, 3, 6 WPI)	5	40	2.166
				8	80	3.327
				10	100	3.227
				12	100	3.042
				35	100	2.476
p1346b	KKK - HBS <sub>19-32</sub> Th -GG- (Seq. ID No.18)	FELLTRILTIPQSLD (Seq. ID No.15)	CFA/IFA (0, 3, 6 WPI)	5	40	3.690
				8	60	3.784
				10	60	3.628
				12	60	3.580
				35	20	3.879

Table 3 (continued)

p1345c	Inv - GG - KKK - HBS <sub>19-32</sub> Th -GG (Seq. ID No.19)	FELLTRILTIPOSLD (Seq. ID No.15)	CFA/IFA (0, 3, 6 WPI)	5	80	3.755
				8	80	4.141
p2134b	HBC <sub>30-69</sub> - GG - Somatostatin (Seq. ID No.8)	SDFEPSVRDLLDTASALY RE (Seq. ID No.4)	0.4% Alum 0, 2, 4, WPI	10	100	3.428
				12	80	3.770
				35	80	3.166
				4	75	3.00
p2134c	Inv-GG-HBC <sub>30-69</sub> -GG- Somatostatin (Seq. ID No.25)	SDFEPSVRDLLDTASALY RE (Seq. ID No.4)	0.4% Alum 0, 2, 4, WPI	6	100	3.22
				8	100	3.18
				4	75	2.77
				6	100	2.98
p2134d	Somatostatin-GG-HBC <sub>30-69</sub> -GG-Inv (Seq. ID No.26)	SDFEPSVRDLLDTASALY RE (Seq. ID No.4)	0.4% Alum 0, 2, 4, WPI	8	100	2.84
				4	0	-
				6	0	-
				8	0	-
p2135e	Somatostatin-GG-TT <sub>615-631</sub> -GG-Inv (Seq. ID No.13)	WVRDIIDDFTESSQKT (Seq. ID No.5)	0.4% Alum 0, 2, 4, WPI	6	100	2.80

\*Sequence of Somatostatin: AGCKNFEWKTFTSC (Seq. ID No.1)

<sup>b</sup>Sequence of Inv (i.e. invasin adjuvant domain): TAKSKKFPSTATYQF (Seq. ID No.2)

<sup>c</sup> Test results for pooled sera from ELISA-reactive animals.

Table 4

Amino Acid Sequences of Pathogen-derived Th Epitopes

Description of Th	SEQ ID NO:	Amino Acid Sequence
HBC <sub>50-69</sub> Th	4	SDFFPSVRDLLDTASALYRE
TT <sub>615-631</sub> Th	5	WVRDIIDDFTNESQKT
PT <sub>149-176</sub> Th	6	KKLRRLLYMIYMSGLAVRVHVSKEEQYY DY
HBS <sub>19-32</sub> Th	15	FFLLTRILTIPQSLD
MVF <sub>258-277</sub> Th	21	GILES RGIKARITHVDTESY
CTA <sub>8106-130</sub> Th	23	ALNIWDREFDVSTLGATSGYLKGNS
CTP <sub>11</sub> Th	27	TINKPKGYVGKE
DT <sub>1</sub> Th	28	DSETADNLEKTVAALSILPGHG
MVF <sub>1</sub> Th	29	LSEIKGVIVHRLEGV

-30-

Table 5

## Amino Acid Sequences of Artificial Th Epitopes

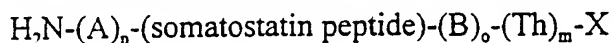
Description of Th	SEQ ID NO:	Amino Acid Sequence
Syn Th (1,2,4)	14	KKKIITITRIITIITTID
(1,4,9 PALINDROMIC) Th	7	ISEIKGVIVHKIEGI MT RT TRM TM L L V
(1,4,9 PALINDROMIC) simplified Th	30	ISEIKGVIVHKIEGI T RT TR T
IS(1,4,9 PALINDROMIC)LF simplified Th	31	ISISEIKGVIVHKIEGILF T RT TR T

CLAIMS

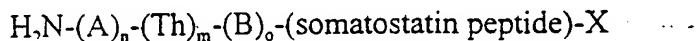
We claim:

1. A peptide conjugate comprising a helper T cell epitope sequence (Th) covalently attached to somatostatin or a crossreactive and immunologically functional analog thereof.

2. A peptide conjugate of Claim 1 wherein said peptide conjugate is represented by the formula



or



wherein

H<sub>2</sub>N is the N-terminal α-NH<sub>2</sub> of the peptide conjugate,

each A is independently an amino acid or a general immunostimulatory sequence;

each B is chosen from the group consisting of amino acids, -NHCH(X)CH<sub>2</sub>SCH<sub>2</sub>CO-,  
-NHCH(X)CH<sub>2</sub>SCH<sub>2</sub>CO(ε-N)Lys-,  
-NHCH(X)CH<sub>2</sub>S-succinimidyl(ε-N)Lys-, and  
-NHCH(X)CH<sub>2</sub>S-(succinimidyl)-;

each Th is independently a sequence of amino acids that comprises a helper T cell epitope, or an immune enhancing analog or segment thereof;

somatostatin peptide is somatostatin or a crossreactive and immunologically functional analog thereof;

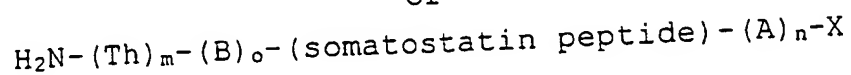
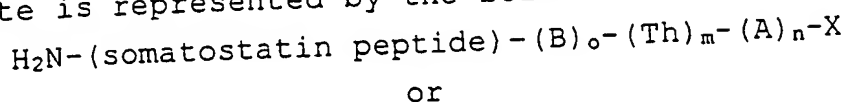
X is an amino acid α-COOH or α-CONH<sub>2</sub>;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

3. A peptide conjugate of Claim 1 wherein said peptide conjugate is represented by the formula



wherein

H<sub>2</sub>N is the N-terminal α-NH<sub>2</sub> of the peptide conjugate,

each A is independently an amino acid or a general immunostimulatory sequence;

each B is chosen from the group consisting of amino acids, -NHCH(X)CH<sub>2</sub>SCH<sub>2</sub>CO-,  
-NHCH(X)CH<sub>2</sub>SCH<sub>2</sub>CO(ε-N)Lys-,  
-NHCH(X)CH<sub>2</sub>S-succinimidyl(ε-N)Lys-, and  
-NHCH(X)CH<sub>2</sub>S-(succinimidyl)-;

each Th is independently a sequence of amino acids that comprises a helper T cell epitope, or an immune enhancing analog or segment thereof;

somatostatin peptide is somatostatin or a crossreactive and immunologically functional analog thereof;

X is an amino acid α-COOH or α-CONH<sub>2</sub>;

n is from 1 to about 10;

m is from 1 to about 4; and

o is from 0 to about 10.

4. A peptide conjugate of claim 2 or claim 3, wherein each B is chosen from the group consisting of natural and unnatural amino acids.

5. A peptide conjugate of any one of claims 1-4 wherein said somatostatin peptide is somatostatin.

6. A peptide conjugate of any one of claims 1-4 wherein said Th is an SSAL epitope.

7. A peptide conjugate of any one of claims 1-4 wherein said Th has an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 SEQ ID NO:30, and SEQ ID NO:31.

8. A peptide conjugate of claim 2 wherein said peptide conjugate has an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

9. A peptide conjugate of claim 2 or claim 3 wherein at least one A is an invasin domain.

10. A peptide conjugate of claim 2 wherein n is 3, and (A)<sub>3</sub> is (invasin domain)-Gly-Gly.

11. A peptide conjugate of claim 9 or claim 10 wherein said invasin domain has the amino acid sequence of SEQ ID NO:2.

12. A synthetic peptide of about 25 to about 90 amino acids, which comprises the amino acid sequences of

(a) an invasin domain,

(b) a helper T cell (Th) epitope, and

(c) somatostatin or a crossreactive and immunologically functional analog thereof.

13. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:19 and SEQ ID NO:25.

14. A peptide or peptide conjugate of any one of claims 1 to 13 wherein said peptide stimulates an immune response to somatostatin in a mammal.

15. A peptide or peptide conjugate of claim 14 wherein immunization of a mammal with said peptide conjugate causes a reduction in somatostatin levels in said mammal.

16. A pharmaceutical composition comprising an immunologically effective amount of a peptide or peptide conjugate of any one of claims 1-15, and a pharmaceutically acceptable carrier.

17. A pharmaceutical composition of claim 16, wherein said immunologically effective amount of said peptide or peptide conjugate is about 0.5  $\mu$ g to about 1 mg per kilogram body weight per dose.

18. A method for inducing anti-somatostatin antibody production in a mammal which comprises administering to said mammal a pharmaceutical composition of claim 16 or claim 17.

19. A method for increasing growth rate in a mammal which comprises administering a pharmaceutical composition of claim 16 or claim 17 to said mammal.

20. A method of increasing growth rate in a mammal which comprises administering to a mammal an amount of a

pharmaceutical composition of claim 16 or claim 17 sufficient to reduce somatostatin levels.

21. A composition comprising a mixture of two or more peptides or peptide conjugates of any one of claims 1-15.

22. A pharmaceutical composition comprising an immunologically effective amount of a composition of claim 21 and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition of claim 22, wherein said immunologically effective amount of said composition is about 0.5  $\mu$ g to about 1 mg per kilogram body weight per dose.

24. A method for inducing anti-somatostatin antibody production in a mammal which comprises administering to said mammal a pharmaceutical composition of claim 22 or claim 23.

25. A method for increasing growth rate in a mammal which comprises administering a pharmaceutical composition of claim 22 or claim 23 to said mammal.

26. A method of increasing growth rate which comprises administering to a mammal an amount of a pharmaceutical composition of claim 22 or claim 23 sufficient to reduce somatostatin levels.

27. A branched polymer comprising a lysine, trilycine, or heptalysine core, covalently attached to two, four, or eight peptide conjugates, respectively, of any one of claims 1-15.

28. A polymer comprising one or more peptide conjugates of any one of claims 1-3 and claims 5-15, cross-linked by

a bifunctional crosslinking agent.

29. A Th epitope peptide selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 SEQ ID NO:30, and SEQ ID NO:31.

(i) APPLICANT: UNITED BIOMEDICAL INC.

(ii) TITLE OF INVENTION: SYNTHETIC SOMATOSTATIN IMMUNOGEN FOR GROWTH PROMOTION IN FARM ANIMALS

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Morgan & Finnegan  
(B) STREET: 345 Park Avenue  
(C) CITY: New York  
(D) STATE: NY  
(E) COUNTRY: USA  
(F) ZIP: 10154-0054

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TBA  
(B) FILING DATE: 18-JUNE-1999  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: MARIA C.H. LIN  
(B) REGISTRATION NUMBER: 29,323  
(C) REFERENCE/DOCKET NUMBER: 1151-4155PC1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 212-758-4800  
(B) TELEFAX: 212-751-6849

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr  
1 5 10  
Ser Cys

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala  
1 5 10  
Thr Tyr Gln Phe  
15

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Pro Pro Xaa Pro Xaa Pro  
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
1                               5          10
Thr Ala Ser Ala Leu Tyr Arg Glu
      15          20

```

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 17 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Val Arg Asp Ile Ile Asp Asp Phe Thr Asn Glu  
1 5 10  
Ser Ser Gln Lys Thr  
15

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Lys Leu Arg Arg Leu Leu Tyr Met Ile Tyr Met  
1 5 10  
Ser Gly Leu Ala Val Arg Val His Val Ser Lys Glu  
15 20  
Glu Gln Tyr Tyr Asp Tyr  
25 30

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: /note= "Ile, Met or Leu"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 2  
(D) OTHER INFORMATION: /note= "Ser or Thr"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 4  
(D) OTHER INFORMATION: /note= "Ile or Arg"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 5  
(D) OTHER INFORMATION: /note= "Lys or Thr"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site  
(B) LOCATION: 10

(D) OTHER INFORMATION: /note= "His or Thr"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 11

(D) OTHER INFORMATION: /note= "Lys or Arg"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 12

(D) OTHER INFORMATION: /note= "Ile, Met or Leu"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 14

(D) OTHER INFORMATION: /note= "Gly or Thr"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 15

(D) OTHER INFORMATION: /note= "Ile, Met or Val"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Xaa	Xaa	Glu	Xaa	Xaa	Gly	Val	Ile	Val	Xaa	Xaa	Xaa
1				5					10		
Glu	Xaa	Xaa									
				15							

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser	Asp	Phe	Phe	Pro	Ser	Val	Arg	Asp	Leu	Leu	Asp
1				5					10		
Thr	Ala	Ser	Ala	Leu	Tyr	Arg	Glu	Gly	Gly	Ala	Gly
			15				20				
Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr	Ser	Cys
25					30					35	

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys	Lys	Leu	Arg	Arg	Leu	Leu	Tyr	Met	Ile	Tyr	Met
1				5					10		
Ser	Gly	Leu	Ala	Val	Arg	Val	His	Val	Ser	Lys	Glu
		15					20				
Glu	Gln	Tyr	Tyr	Asp	Tyr	Gly	Gly	Ala	Gly	Cys	Lys
25				30						35	
Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr	Ser	Cys		
			40					45			

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ix) FEATURE:

(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "Ile, Met or Leu"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2

(D) OTHER INFORMATION: /note= "Ser or Thr"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 4

(D) OTHER INFORMATION: /note= "Ile or Arg"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: /note= "Lys or Thr"

- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 10  
    (D) OTHER INFORMATION: /note= "His or Thr"
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 11  
    (D) OTHER INFORMATION: /note= "Lys or Arg"
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 12  
    (D) OTHER INFORMATION: /note= "Ile, Met or Leu"
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 14  
    (D) OTHER INFORMATION: /note= "Gly or Thr"
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 15  
    (D) OTHER INFORMATION: /note= "Ile, Met or Val"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa	Xaa	Glu	Xaa	Xaa	Gly	Val	Ile	Val	Xaa	Xaa	Xaa
1				5					10		
Glu	Xaa	Xaa	Gly	Gly	Ala	Gly	Cys	Lys	Asn	Phe	Phe
		15					20				
Trp	Lys	Thr	Phe	Thr	Ser	Cys					
25					30						

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 31 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 17  
    (D) OTHER INFORMATION: /note= "Ile, Met or Leu"
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 18  
    (D) OTHER INFORMATION: /note= "Ser or Thr"
- (ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 21  
    (D) OTHER INFORMATION: /note= "Ile or Arg"

- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 22  
 (D) OTHER INFORMATION: /note= "Lys or Thr"
- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 26  
 (D) OTHER INFORMATION: /note= "His or Thr"
- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 27  
 (D) OTHER INFORMATION: /note= "Lys or Arg"
- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 28  
 (D) OTHER INFORMATION: /note= "Ile, Met or Leu"
- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 30  
 (D) OTHER INFORMATION: /note= "Gly or Thr"
- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 31  
 (D) OTHER INFORMATION: /note= "Ile, Met or Val"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala	Gly	Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr
1				5					10		
Ser	Cys	Gly	Gly	Xaa	Xaa	Glu	Ile	Xaa	Xaa	Val	Ile
		15					20				
Val	Xaa	Xaa	Xaa	Glu	Xaa	Xaa					
25					30						

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 51 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala	Gly	Cys	Lys	Asn	Phe	Phe	Trp	Lys	Thr	Phe	Thr
1				5					10		
Ser	Cys	Gly	Gly	Trp	Val	Arg	Asp	Ile	Ile	Asp	Asp
		15					20				
Phe	Thr	Asn	Glu	Ser	Ser	Gln	Lys	Thr	Gly	Gly	Thr
25				30					35		
Ala	Lys	Ser	Lys	Lys	Phe	Pro	Ser	Tyr	Thr	Ala	Thr

**SUBSTITUTE SHEET (RULE 26)**

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 38 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
1          5          10
Thr Tyr Gln Phe Gly Ile Leu Glu Ser Arg Gly Ile
          15          20
Lys Ala Arg Ile Thr His Val Asp Thr Glu Ser Tyr
25          30          35
Gly Gly

```

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

Lys Lys Lys Phe Phe Leu Leu Thr Arg Ile Leu Thr
1          5          10
Ile Pro Gln Ser Leu Asp Gly Gly
          15          20

```

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 38 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
1          5          10
Thr Tyr Gln Phe Gly Gly Lys Lys Lys Phe Phe Leu
          15          20
Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp
25          30          35
Gly Gly

```

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

Lys Lys Lys Ile Ile Thr Ile Thr Arg Ile Ile Thr
1           5           10
Ile Ile Thr Thr Ile Asp Gly Gly Ala Gly Cys Lys
          15           20
Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys
25           30

```

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile
1           5           10
Thr His Val Asp Thr Glu Ser Tyr
          15           20

```

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```

Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr
1           5           10
Ser Cys Gly Gly Gly Ile Leu Glu Ser Arg Gly Ile
          15           20
Lys Ala Arg Ile Thr His Val Asp Thr Glu Ser Tyr
25           30           35

```

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Ser
 1             5             10
Thr Leu Gly Ala Thr Ser Gly Tyr Leu Lys Gly Asn
      15             20
Ser
25

```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 41 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Ser
 1             5             10
Thr Leu Gly Ala Thr Ser Gly Tyr Leu Lys Gly Asn
      15             20
Ser Gly Gly Ala Gly Cys Lys Asn Phe Phe Trp Lys
25             30             35
Thr Phe Thr Ser Cys
      40

```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 54 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```

Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr Thr Ala
 1             5             10
Thr Tyr Gln Phe Gly Gly Ser Asp Phe Phe Pro Ser
      15             20
Val Arg Asp Leu Leu Asp Thr Ala Ser Ala Leu Tyr
25             30             35
Arg Glu Gly Gly Ala Gly Cys Lys Asn Phe Phe Trp
      40             45
Lys Thr Phe Thr Ser Cys
      50

```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 54 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```

Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr
1           5           10
Ser Cys Gly Gly Ser Asp Phe Phe Pro Ser Val Arg
          15           20
Asp Leu Leu Asp Thr Ala Ser Ala Leu Tyr Arg Glu
25           30           35
Gly Gly Thr Ala Lys Ser Lys Lys Phe Pro Ser Tyr
          40           45
Thr Ala Thr Tyr Gln Phe
50

```

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

Thr Ile Asn Lys Pro Lys Gly Tyr Val Gly Lys Glu
1           5           10

```

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```

Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Val
1           5           10
Ala Ala Leu Ser Ile Leu Pro Gly His Gly
          15           20

```

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu  
1                      5                      10  
Glu Gly Val  
                    15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Ser or Thr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "Lys or Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Gly or Thr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 10
- (D) OTHER INFORMATION: /note= "His or Thr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "Lys or Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 14
- (D) OTHER INFORMATION: /note= "Gly or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ile Xaa Glu Ile Xaa Xaa Val Ile Val Xaa Xaa Ile  
1                      5                      10  
Glu Xaa Ile  
                    15

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 4  
(D) OTHER INFORMATION: /note= "Ser or Thr"

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 7  
(D) OTHER INFORMATION: /note= "Lys or Arg"

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 8  
(D) OTHER INFORMATION: /note= "Gly or Thr"

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 12  
(D) OTHER INFORMATION: /note= "His or Thr"

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 13  
(D) OTHER INFORMATION: /note= "Lys or Arg"

- (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 16  
(D) OTHER INFORMATION: /note= "Gly or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Ile Ser Ile Xaa Glu Ile Xaa Xaa Val Ile Val Xaa
1           5           10
Xaa Ile Glu Xaa IleLeu Phe
15
```

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32

```
Lys Lys Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile
1           5           10
```

Gly Ile Thr Glu Leu  
15

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 22 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Lys Lys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu  
1 5 10  
Arg Val Pro Lys Val Ser Ala Ser His Leu  
15 20

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Tyr Asp Pro Asn Tyr Leu Arg Thr Asp Ser Asp Lys  
1 5 10  
Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn  
15 20  
Arg Ile Lys  
25

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Ala Tyr Ala Arg Cys Pro Asn Gly Thr Arg Ala  
1 5 10  
Leu Thr Val Ala Glu Leu Arg Gly Asn Ala Glu Leu  
15 20

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Val	Ser	Phe	Gly	Val	Trp	Ile	Arg	Thr	Pro	Pro	Ala
1				5					10		
Tyr	Arg	Pro	Pro	Asn	Ala	Pro	Ile	Leu			
		15				20					

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Pro	His	His	Thr	Ala	Leu	Arg	Gln	Ala	Ile	Leu	Cys
1				5					10		
Trp	Gly	Glu	Leu	Met	Thr	Leu	Ala				
		15				20					

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg	Ala	Gly	Arg	Ala	Ile	Leu	His	Ile	Pro	Thr	Arg
1				5					10		
Ile	Arg	Gln	Gly	Leu	Glu	Arg					
		15									

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu Val  
 1 5 10  
 Leu Gln Arg Ala Gly Arg Ala Ile Leu  
 15 20

## (2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 39 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser Ser  
 1 5 10  
 Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu  
 15 20  
 Leu Val Asp Ile Gly Phe Ala Ala Thr Asn Phe Val  
 25 30 35  
 Glu Ser Cys

## (2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala  
 1 5 10  
 Ser Ser Val Phe Asn Val Val Asn Ser  
 15 20

## (2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 17 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Lys Trp Phe Lys Thr Asn Ala Pro Asn Gly Val Asp  
 1 5 10

Glu Lys Ile Arg Ile  
15

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 14 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Leu Gln Gly Lys Ile Ala Asp Ala Val Lys Ala  
1                      5                      10  
Lys Gly

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Leu Ala Ala Gly Leu Val Gly Met Ala Ala Asp  
1                      5                      10  
Ala Met Val Glu Asp Val Asn  
15

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Ser Thr Glu Thr Gly Asn Gln His His Tyr Gln Thr  
1                      5                      10  
Arg Val Val Ser Asn Ala Asn Lys  
15                      20

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/31, 39/00, 39/02, 39/29; C07K 14/65

US CL : 424/ 185.1, 189.1, 190.1, 198.1, 227.1; 514/806; 530/311; 930/160

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/ 185.1, 189.1, 190.1, 198.1, 227.1; 514/806; 530/311; 930/160

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, MEDLINE

search terms: invasin, T helper cell epitope, somatostatin

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SPENCER, G. S. G. Hormonal systems regulating growth, a review. Livestock Production Science. 1985, Vol. 12, pages 31-46, see especially paragraph bridging pages 40-41.	1-28
Y	US 5,759,551 A (LADD et al.) 02 June 1998, Abstract, column 3, lines 30-38; column 4, lines 19-38.	1-29
Y	US 4,812,554 A (RIGGS) 14 March 1989, col. 4, lines 13-53.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

27 AUGUST 1999

Date of mailing of the international search report

25 OCT 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DAVID ROMEO

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/13923

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out; specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-17 and 21-23, drawn to a peptide conjugate comprising an invasin domain, a helper T cell epitope, and somatostatin.

Group II, claim(s) 18-20, 24, 25 and 26, drawn to a method of inducing anti-somatostatin antibodies and/or increasing growth rate in an animal with a peptide conjugate comprising an invasin domain, a helper T cell epitope, and somatostatin.

Group III, claim(s) 27 and 28, drawn to a branched polymer of a peptide conjugate comprising an invasin domain, a helper T cell epitope, and somatostatin.

Group IV, claim(s) 29, drawn to a Th epitope peptide.

With respect to unity of invention PCT Rule 13.1 states:

The international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention").

ly, PCT Rule 13.2 states:

Where a group of inventions is claimed in one and the same international application, the requirement of unity of invention referred to in Rule 13.1 shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

ed to the application of PCT Rule 13, 37 CFR 1.475 concerning unity of invention states:

1) An international and a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in an application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

Groups I-IV recite a plurality of products and methods of using same. The inventive concept joining these groups appears to be an immunogenic conjugate of somatostatin comprising an invasin domain, a helper T cell epitope, and somatostatin. In order for these inventions to have unity of invention it is necessary that the single inventive concept be a contribution over the prior art. However, SPENCER teaches growth promotion in animals by immunization with somatostatin. See the paragraph bridging pages 40-41. LADD et al. teach conjugation of a hormone with a helper T cell epitope and an invasin domain in order to induce an early and strong immune response (column 3, lines 30-38). It would have been obvious to one of ordinary skill in the art to combine these teachings for the purpose of growth promotion in farm animals with a reasonable expectation of success. Therefore, the inventions of groups I-IV do not fulfill the requirements of unity of invention with respect to the products or the methods of using same.

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